REPORT TITLE

Toxicology Response by the Endosulfan Task Force to the Health Effects Division Risk Assessment for the Endosulfan Reregistration Eligibility Decision Document Dated February 17, 2000:

Selection of a Dermal No Observable Adverse Effect Level (NOAEL) for Endosulfan

DATA REQUIREMENT

Not Applicable

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SUBMISSION VOLUME

Volume 1 of 3

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

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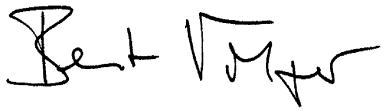
Signature: _____

Date: January 4, 2001

STATEMENT OF GOOD LABORATORY PRACTICE

The following response is not subject to the principles of 40 CFR 160, Good Laboratory Practice Standards, as promulgated in Federal Register, 54, No. 158, 34067-34704, August 17, 1989.



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Date: January 4, 2001

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HEALTH EFFECTS DIVISION (HED) RISK ASSESSMENT FOR THE ENDOSULFAN REREGISTRATION ELIGIBILITY DECISION DOCUMENT, DATED FEBRUARY 17, 2000

TOXICOLOGY CHAPTER

RE: Endosulfan: HED Risk Assessment for the Endosulfan RED Document (DP Barcode: D250471; Memo by Stephen C. DeVito, Ph.D., dated February 17, 2000) - Exposure Assessment, Section 3.0 "Hazard Characterization" and Related Documents;

Endosulfan079401: Toxicology Chapter for the Reregistration Eligibility Document (HED memo by Nicole C. Paquette, Ph.D. dated November 22, 1999.

The Endosulfan Task Force (ETF), comprised of Aventis CropScience, FMC, and Makhteshim-Agan North America, respectfully submit the following three volumes in response to the above referenced draft chapter. There are three key areas of concern regarding the EPA's review of the endosulfan toxicity data that the ETF will address. These areas are:

- The NOAEL selection for the 21-day dermal study in rats (Volume 1)
- Requirement of a developmental neurotoxicity study and retention of a FQPA safety factor of 3x due to uncertainty associated with this data gap (Volume 2)
- EPA's suggestion that endosulfan may be an endocrine disruptor (Volume 3)

This volume specifically addresses the selection of an appropriate dermal NOAEL for the assessment of risk to workers and residential application and post-application exposures.

I. INTRODUCTION

In preparation for the final Reregistration Eligibility Decision (RED) on the active ingredient endosulfan, the EPA Health Effects Division (HED) provided the Endosulfan Task Force (ETF) with a draft of their human health risk assessment for all registered uses of this chemical. Supporting documents for this risk assessment included the Hazard Identification Assessment Review Committee (HIARC) Toxicology Chapter, the HIARC report on toxicological endpoints for risk assessment, and the FQPA Safety Factor Committee report. On May 10, 2000, the ETF submitted an initial 30-day response identifying errors in the draft risk assessment and providing brief summaries on issues of concern regarding the selection of toxicological endpoints, application of FQPA safety factors and implications regarding the potential of endosulfan to be an endocrine disruptor.

The purpose of this submission is to further elucidate the areas of concern discussed briefly in the 30-day response. One of the most significant issues to be discussed by the ETF is the selection of the dermal NOAEL for use in worker and residential exposure scenarios and risk assessments. The ETF concurs with the HIARC's selection of the 21-day dermal toxicity study

for providing the most appropriate data for dermal exposure assessments. However, the ETF does not agree with the HIARC's determination of the No Observable Adverse Effect Level (NOAEL) resulting from its review of the available data. The remainder of this document will provide a detailed review of the available data, a weight-of-evidence evaluation of the dermal toxicity of endosulfan, and a full rationale for selection of an appropriate toxicity endpoint for dermal exposure assessments.

II. NOAEL selection for the 21-day dermal study in rats

A. EPA Conclusion

The EPA HED chapter provided the following conclusion on selection of a dermal toxicity endpoint for dermal exposure assessments. "*The endpoint for the short-term, intermediate- and long-term dermal exposure assessment was based on hepatotoxicity seen in a 21-day study in which endosulfan was applied dermally to rats (NOAEL = 3.0 mg/kg bw/day).*"

The HED selection was based on the HIARC report (ENDOSULFAN 079401: Toxicology Chapter for the Reregistration Eligibility Document. Nicole Paquette memo dated 2/9/00). The HIARC report concluded that "*for systemic toxicity, the NOAEL was 3 mg/kg bw/day and the LOAEL was 9 mg/kg bw/day based on increased mortality in males, and increased liver abnormalities (enlargement of parenchymal cells, loss of cytoplasmic basophilia and isolated cell necrosis and frequent mitosis) in both sexes.*"

In addition, the HIARC made this comment in support of their endpoint selection. "*The dermal NOAEL of 3 mg/kg bw/day is supported by the dermal equivalent dose of 4 mg/kg bw/day obtained by the use of the dermal absorption factor (45%) in conjunction with the oral NOAEL (2.0 mg/kg bw/day) established in the developmental toxicity study in rats (2 mg/kg bw/day ÷ 0.45 = 4 mg/kg bw/day).*" *(p.9)*

B. ETF Response

The ETF does not agree with the HIARC assessment of the 21-day dermal study NOAEL. The ETF believes that the most accurate assessment should be made based on the dermal toxicity database in its entirety. Therefore, ETF evaluated all of the available dermal toxicity studies and has provided a weight-of-evidence determination on the most appropriate NOAEL for use as an endpoint in dermal exposure assessments. The ETF believes that the most appropriate NOAEL is 12 mg/kg bw/day, based on increased mortality in female rats. This determination is well supported by all of the dermal toxicity studies, as well available information from subchronic oral studies. The following is an overview of this assessment.

To examine the potential toxicity of endosulfan technical on repeated dermal exposure, two subchronic 21-day dermal toxicity studies on rats have been conducted according to EPA guidelines (MRID 00146841 & 00147744). There is also a non-guideline study in the public literature (Dikshith et al. 1988, Appendix 3), which, while inadequate with

respect to guideline requirements, is supportive of the findings from the two previously mentioned guideline studies. In addition, there are two 21-day dermal toxicity studies using formulated endosulfan. The Agency has reviewed the first study with formulated endosulfan, 49.5% purity in a wettable powder (MRID# 41048506), and a fourth study has been submitted which used an emulsifiable concentrate formulation at 33.3% purity (MRID# 41048505). While these formulated product studies cannot be directly correlated to the results from technical material studies, they can provide supportive evidence for selection of a dermal toxicity endpoint. Detailed summaries of all five dermal toxicity studies are appended in this report (Appendices 1-5).

Study Number	MRID	Formulation	Doses (mg/kg bw/day)	
			Male	Female
A30754	00147744	Technical	0, 12, 48, 96, 192	0, 3, 6, 12, 48
A30753	00146841	Technical	0, 1, 3, 9, 27, 81	0, 1, 3, 9, 27
A41365	Dikshith, T.S.S.	Technical	18.8, 37.5, 62.5	9.83, 19.7, 32.0
A39426	41048506	50WP	0, 40, 160, 640	0, 40, 80, 160
A39279	41048505	3EC	0, 0, 27, 54, 81	0,0, 9, 12, 18, 36

Following evaluation of the key toxicity endpoints of concern, the NOAEL selected should be based on consistency of effects across studies. Effects noted in most of the studies included clinical symptoms of intoxication and increased incidence of mortality, with the female rat being significantly more sensitive than the male. In selecting the dermal exposure NOAEL, HIARC referred to the two *male* deaths, in the second 21-day dermal toxicity study (MRID 00146841) using technical endosulfan, which occurred at 9-mg/kg bw/day. HIARC also cited liver histopathological changes starting at 9 mg/kg bw/day. HIARC supported their NOAEL selection by comparing the dermal NOAEL of 3 mg/kg bw/day to the dermally equivalent NOAEL of 4 mg/kg bw/day (oral NOAEL of 2 mg/kg bw/day ÷ dermal absorption factor of 45%) from the developmental toxicity study. The common endpoint for this comparison was increased mortality. The ETF addressed both increased incidences of mortality and liver histopathological changes in the review of the available data. The ETF does not concur with HIARC's selection of this NOAEL based on either mortality in males or liver histopathology.

1. Increased Incidence of Mortality

The ETF does not believe that the male deaths noted at 9 mg/kg bw/day in the 21-day dermal toxicity study (MRID 00146841) are treatment-related, nor is comparison to the female mortality incidence in the developmental toxicity study appropriate or supportive of the HIARC NOAEL.

a. Significance of Male Deaths at 9 mg/kg bw/day

- As described by the pathologist report in the 21-day dermal study (MRID 00146841), necropsy conducted on the two male rats (nos. 23 & 24) that died in the 9 mg/kg bw/day dose group revealed evidence of a pre-existing, non-treatment-related developmental disturbance. The first male's testes, spleen and

thymus were significantly reduced in size (immature) with no evidence of inflammation and/or atrophy. The second male also showed marked reduction in size of the testes, seminal vesicles and liver, without signs of inflammation or atrophy. These pre-existing deficiencies compromised the animals' ability to thrive, and the resulting deaths should not be considered in the overall toxicological evaluation for endosulfan. Furthermore, no mortalities were seen in male rats at the next highest dose of 27 mg/kg bw/day, and in the other four dermal studies the lowest dose that produced mortality in male rats was 81 mg/kg bw/day. The lack of a clear dose-response within and between studies strongly suggests a non-treatment-related effect.

- Evaluation of body weight gain, a sensitive indicator of general health, shows that both males were already demonstrating some growth retardation prior to and including the first dosing period.

Table 1: Body Weight Gain for Male Rats at 9 mg/kg bw/day

Animal Number	Body Weight (g) Day – 4 of Study	Body Weight Gain (g) Day +1 of Study	Body Weight Gain (g) Day +1 of Study	Food Consumption (g/day) Day +1 of Study
00019	182	203	21	17.4
00020	168	205	37	21.6
00021	171	212	41	21.2
00022	179	192	13	15.8
00023	**176**	**184**	**8**	**16.4**
00024	**175**	**184**	**9**	**17.8**

b. Comparison to Female Mortality in the Developmental Study

- Comparison of the NOAEL from the developmental toxicity study (increased mortality in *female* rats) to the HIARC selected NOAEL from the dermal study (deaths of two *male* rats) is not toxicologically appropriate. First, as stated previously, the deaths of the two male rats at 9 mg/kg bw/day was primarily due to an underlying developmental deficiency which would have compromised their ability to thrive even under normal conditions. Second, both dermal and oral exposure studies have shown that the female rat response to endosulfan toxicity is significantly different than that of male rats, with female rats being more sensitive by all routes of exposure. Lastly, females under reproductive stress often demonstrate unique sensitivity to toxic insult due to the significant metabolic and biological changes brought on by pregnancy. Therefore, interpreting and extrapolating endpoints of toxicity from this type of study to other non-reproductive type studies, as well as comparisons between sexes, incorporates too many confounding factors and should be not considered the most appropriate type of comparison.
- Even if effects from the developmental study are taken into consideration, the comparison of NOAELs from different routes of exposure is not an accurate science. NOAELs are an artifact of dose selection and can range significantly within and between similar studies of the same route of exposure. Therefore,

several points can be made regarding extrapolation of the NOAEL from the developmental toxicity study in support of the dermal NOAEL selection:

1) Assumption of a dermal absorption factor of 45% is worst case (associated with the lowest dose applied of 0.1 mg/kg) and does not take into account the non-linear kinetics noted in the dermal absorption study (see Appendix 6 regarding interpretation of an appropriate dermal absorption factor). Based on the doses applied in the dermal study the more appropriate absorption rate would be 20%, derived from the 10 mg/kg dose group in the absorption study. The resulting dermally equivalent dose would be 10mg/kg bw/day from the developmental study (2 mg/kg/day ÷ 20% abs);
2) The actual NOAEL in the developmental study probably lies somewhere between 2 and 6 mg/kg bw/day (the lowest dose and the lowest effect level), showing a possible range for the dermally equivalent NOAEL of 10 to 30 mg/kg bw/day, which encompasses the ETF recommended dermal NOAEL of 12 mg/kg bw/day; and
3) Significant mortality in females noted in the developmental study was not supported by data from the reproductive study where doses of 6.2 mg/kg bw/day for up to 270 days of exposure (prior to, during and after pregnancy) did not produce mortality.

All of these factors result in significant uncertainty surrounding the extrapolation of a dermal NOAEL to an oral NOAEL and suggest that a fairly broad range of NOAELs could be supported by the available toxicity data. In fact, a review of subchronic oral and dermal studies in which increased mortality is the endpoint of concern show No Effect Levels ranging from 2 mg technical/kg/day up to 32 mg technical/kg/day for female rats. This includes the reproductive toxicity study and the 13-week feeding study, where no mortality occurred in females at doses of 6.2 mg technical/kg/day and 18 mg technical/kg/day, respectively.

- Since the endpoint of concern is risk resulting from potential dermal exposure to humans, and human skin has been shown to be on average 4 times less permeable to endosulfan than rat skin (see Appendix 6), direct extrapolation of a dermal NOAEL from the 21-day dermal study is the most appropriate method for determining a toxicity endpoint for dermal exposure.

- Therefore, the most sensitive endpoint from the dermal toxicity studies would be increased incidence of mortality in female rats. The NOAEL for increased mortality in females in the dermal study referenced by EPA was 12 mg/kg bw/day. Evaluation of the other four dermal toxicity studies supports a NOAEL of 12 mg/kg bw/day or higher for increased incidence of female mortality.

Table 2: Mortality Incidence in Two 21-Day Dermal Toxicity Studies

	Females								Males									
	Dose (mg/kg bw/day)								Dose (mg/kg bw/day)									
Study 1	0		3	6		12		48	0				12		48		96	192
Study 2	0	1	3		9		27		0	1	3	9		27		81		
Mortalities[a]	0	0	1[a]	1[a]	0	1[a]	5	4	0	0	0	2[b]	0	0	0	3	0	2
Hypersalivation						1[a]		4								1		3
Convulsions								1								1		3

Non-substance-related findings:

[a] death / symptom occurred on day 18 of the study due to inappropriate bandaging of the rats causing self-inflicted wounds and death.

[b] these two male deaths were associated with non-substance-related developmental disturbances (very small immature testes and livers)

In Dikshith et al., females were dosed dermally for 30 days at 0, 9.83, 19.66 and 32.0 mg/kg bw/day. There were no reported deaths in this study. Therefore, the NOAEL for increased mortality in females for this study was 32 mg/kg bw/day, the highest dose tested. In the two 21-day dermal toxicity studies using formulated endosulfan, significantly increased mortality in female rats was noted at 160 mg formulation/kg bw/day (50 WP) and 36 mg formulation/kg bw/day (3 EC). These doses roughly equate to effect levels for technical material of 80 mg/kg bw/day and 12 mg/kg bw/day, with resulting NOAELs from these studies, for increased mortality of females, of 40 mg/kg bw/day and 6 mg/kg bw/day, respectively. As expected, the EC formulation study produced a slightly lower NOAEL than the rest of the studies, due to solvent-enhanced dermal absorption. However, while this study was acknowledged by the ETF as part of the total weight-of-evidence, this study is not relevant for worker re-entry or post-application exposure risk assessments. For worker re-entry and post-application exposure the most appropriate data is from technical material since the only exposure would be from dried residues. Therefore, the ETF believes that the overall weight-of-evidence from these five studies clearly support a NOAEL of 12 mg/kg bw/day for increased mortality in females, and that this is the most appropriate endpoint for dermal exposure assessments to set a restricted re-entry interval (REI) for endosulfan.

2. Evidence of Histopathological Changes in Liver

In the study selected by EPA to establish the dermal NOAEL, the Agency also cited liver effects at doses of 9 mg/kg bw/day and up in both sexes. The liver histopathological findings consisted of enlargement of parenchymal cells in peripheral sections, together with loss of cytoplasmic basophilia, isolated cell necroses, and frequent mitosis. However, a thorough review of this study shows that these effects were considered “very slight” by the pathologist, were only seen in a few animals, and were neither sex- nor dose-related.

Table 3: Histopathological Summary of Liver Effects (MRID 00146841)

	Males						Females				
Dose Group	**0**	**1**	**3**	**9**	**27**	**81**	**0**	**1**	**3**	**9**	**27[d]**
Number Examined	6	6	6	6	6	6	6	6	6	6	6
Isolated small round cell infiltrates			5	3	4	4	2	5	4	6	1
Isolated small Kupffer cell infiltrates		4									
Acinoperipheral small-drop fatty changes		2	1	3[b]	6[b]	1				5[a]	6[c]
Cell group necrosis w/ Kupffer cell reaction								1			
Isolated epithelial necrosis				1							
Enlarged acinopheral hepatocytes w/ loss of cytoplasmic basophilia				2	2					2	3
Medium-drop fat storage in isolated hepatocytes						2					
Enlargement of isolated acinoperipheral hepatocytes						2					
Isolated mitosis						2					
Increased frequency of mitoses				2							

[a] graded as slight acinoperipheral small-drop fatty change of the parenchyma
[b] graded as slight acinoperipheral small-drop fatty change of the epithelium
[c] graded as very slight small-drop fatty change of isolated hepatocytes
[d] Five of six animals died spontanteously and showed signs of autolytic changes in all organs. These animals showed acute congestion of the blood vessels together with isolated individual and group necroses, frequently accompanied by Kupffer cell nodules or round cell infiltrates. Isolated granulocytes were also detected in these foci.

Based on the above summary of liver effects there is no indication of a dose- or sex-related trend, as was stated by the pathologist in the original report. In addition, a review of the other dermal studies, in some cases with doses significantly higher than in this study, as well as available oral subchronic studies in rats, did not reveal any histopathological changes of significance in the liver. Therefore, the liver effects noted in this study are not of toxicological significance and do not represent a clear adverse effect level.

III. CONCLUSION

Based on the information provided, the ETF believes that the appropriate NOAEL for this study is 12 mg/kg bw/day, based on increased incidence of mortality in females at 27 mg/kg bw/day. The ETF also believes that this is the most appropriate toxicity endpoint for the evaluation of short-term, intermediate- and long-term post-application dermal exposures for endosulfan (e.g. setting an REI).

APPENDIX 1

Ebert, E.; Leist, K.-H.; Kramer, M. (1985a) Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) Testing for Subchronic Dermal Toxicity (21 Applications over 30 Days) in SPF Wistar Rats; Hoechst Pharma Research Toxicology, Germany; Company file No: A30754; Date: 11 Mar 1985a (MRID 00147744)

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Method B.9 on Repeated dose (28 days) toxicity (dermal), OECD Test Guideline 410 (May 1981) and EPA-FIFRA §82-2 (November 1982)
Material and Methods : Groups of 11 male and 11 female Wistar rats were treated dermally with 2 ml/kg bw of endosulfan (purity 97.2%) in sesame oil for 6 hr/day under total occlusion for 30 days, 5 days/week, in total 21 treatments, at doses of 0, 3, 6, 12, 48, 96 or 192 mg/kg bw. The two low doses were given to females only. The 2 high doses were given to males only. After the 6 hr treatment the bandage was removed and the treated skin washed. Behavior and clinical signs were recorded twice daily, examination of the treated skin area was done before the next application, food consumption and body weights were recorded twice weekly, water consumption once weekly. On day 31 of the study 6 rats/group/sex were sacrificed, while the remaining animals were allowed a recovery period of 14 days. After sacrifice standard hematology, clinical chemistry, urinalysis, macroscopic pathology and histology were carried out.
Findings: This study should be evaluated in conjunction with the described above follow-up study. Main findings are summarized in Table A1.
Mortality: Mortalities occurred between day 2 - 22. At 3, 6 and 12 mg/kg bw/day one female died. These deaths were attributed to the application technique employed and for this reason a follow-up study was started (see above). At 48 mg/kg bw/day 4/11 females died on day 2, 4 or 22. At 192 mg/kg, 2/11 males died due to substance related signs (tremors, hypersalivation and tonic-clonic convulsions).
Clinical signs: No clinical signs were seen in any of the males at 96 mg/kg bw/day. At the high dose, tremors and salivation were observed in 3/11 males, two of which died on day 6 and 9. In the females, first clinical signs started at 12 mg/kg bw/day and consisted of pilo-erection, increased salivation and lacrimation. At higher doses blood encrusted snouts and chromodacryorrhoea were observed. In addition, at the highest dose for the female rats (48 mg/kg bw/day) tonic-clonic convulsions were observed in one female.
Irritation: Dryness and desquamation were seen after the 4th application. These signs had reversed after 3 days.
Body weights: No effects were found on body weights or body weight gain.
Food consumption: No differences were observed on food consumption between the groups.
Water consumption: No differences were observed in water consumption between the groups.
Hematology: No statistically significant hematological effects were found.
Clinical chemistry: Various slight changes were observed. None of these was dose related: Cholinesterase activity was reduced in serum of males at 192 ppm. No effect on serum-, erythrocyte-, or brain ChE-activity was seen after the recovery period.
Other parameters: No changes were found in urinalysis, organ weights, or macroscopic pathology or histology.

Table A1: Endosulfan; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex		Males					Females				
Dose	(mg/kg bw/day)	0	12	48	96	192	0	3	6	12	48
Mortalities		0	0	0	0	2	0	1[1]	1[1]	1[1]	4
Hypersalivation		-	-	-	-	3	-	-	-	-	4
Tremors/Convulsions		-	-	-	-	1	-	-	-	-	1
Autoagression[1]		-	-	-	-	-	-	1	1	1	-
Erythrocyte ChE	(U/L)	247	215	178*	237	218	217	207	205	202	223
Serum ChE	(U/L)	428	374	378	381	288*	1298	1209	1193	1339	1022
Brain ChE	(U/kg)	5592	5543	5570	4666*	5198	5190	4875	4521	4972	5189

[1] The self inflicted wounds and subsequent deaths were caused by biting in order to remove the tight bandage around the trunk.
Significantly different from control, *p<0.05, **p<0.01, ***p<0.001

Conclusion: In this 30-day dermal study with 14-day recovery the LOAEL was 48 mg/kg bw/day based on female deaths. Other female deaths, seen at 3, 6 and 12 mg/kg bw/day, are not considered substance related, but was caused by the tight occlusive bandage resulting in self-inflicted wounds and deaths. As a result of these findings the study was repeated with more comfortable bandaging and, based on the results of this study, with adjusted doses. Essentially no cholinesterase inhibition was found in erythrocytes, serum or brain.

APPENDIX 2

Ebert, E., Leist, K.-H., Kramer, M.(1985b) Endosulfan - Active Ingredient Technical (Code: Hoe 002671 0I ZD97 0003) Testing for Subchronic Dermal Toxicity (21 Applications over 30 Days) in Wistar Rats; Hoechst Pharma Research Toxicology, Germany; Company file No: A30753; Date: 22 Feb. 1985 (MRID 00146841)

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Method B.9 on Repeated dose (28 days) toxicity (dermal), with OECD Test Guideline 410 (May 1981) and with EPA-FIFRA §82-2 (November 1982).
Material and Methods: Groups of 6 male and 6 female Wistar rats were treated dermally with 2 ml/kg bw of endosulfan (purity 97.2%) in sesame oil for 6 hr/day under total occlusion for 30 days, 5 days/week, in total 21 treatments, at doses of 0, 1, 3, 9, 27 mg/kg bw or at 81 mg/kg bw (males only). After the 6-hr treatment the bandage was removed and the treated skin washed. Behavior and clinical signs were recorded twice daily, examination of the treated skin area was done before the next application, food consumption and body weights were recorded twice weekly, water consumption once weekly. At day 31 the animals were sacrificed and standard hematology, clinical chemistry, urinalysis, macroscopic pathology and histology was carried out.
Findings: The main findings are summarized in Table A2. This follow-up study should be evaluated in combination with the first study, described below.
Mortality: Mortalities occurred between day 2 - 8. The two male deaths at 9 mg/kg cannot be considered as treatment related due to developmental disturbances originating prior to treatment: Both males had small immature testes and one also had a very small liver, while the liver weight of the other could not be determined due to autolysis. The developmental disturbance in these animals is likely to have reduced the capacity to metabolize the chemical. No male deaths were observed at 27 mg/kg bw/day.
In addition, 5 females at 27 mg/kg died between day 2 and 6 without any signs of toxicity.
Clinical signs: No clinical signs were seen in any female. One of the 3 males that died at the top dose (81 mg/kg) showed signs of typical of endosulfan intoxication: tono-clonic convulsions, increased salivation and increased respiratory rate.
Irritation: At the start of the treatment a slight irritation was seen at all dose levels and the control group. This subsided within a few days.
Body weights: No effects were found on body weights or body weight gain.
Food consumption: There was a marked reduction in food intake in animals that died inter-currently.
Water consumption: There was a marked reduction in water intake in animals that died inter-currently.
Hematology: No hematological effects were found. The slight reduction in thrombocytes, though statistically significant, falls still within the normal range.
Clinical chemistry: Cholinesterase activity was reduced in serum of males treated at 9 ppm and above. This effect is not judged to be of toxicological relevance: The reduction was not found in the females, which are more sensitive for endosulfan toxicity. Furthermore, this effect did not

occur in the first 30-day dermal toxicity study, though higher doses were applied there. In addition, an *in vitro* assay is available indicating that endosulfan is not a primary AChE-inhibitor. In subchronic and chronic feeding studies on endosulfan there was no evidence of cholinesterase inhibition independent of the species tested (mouse, rat or dog). In addition, in workers producing or applying endosulfan, cholinesterase inhibition has never been found. There is consistency in both dermal subchronic studies about the absence of erythrocyte acetyl-cholinesterase. Therefore, the serum cholinesterase inhibition found only in the second study must have been a spontaneous finding. This effect is not evaluated by WHO as an adverse effect. The change in brain cholinesterase activity was within the normal variation for rats of this age, was not dose-related, and was not consistent with other endosulfan dermal studies. All other serum clinical chemical parameters were also within normal range
Organ weights: All organ weights, including spleen weights with a slight statistical difference in females only, were within a normal range of biological variation.
Urinalysis: No changes were found.
Macroscopic pathology or histology: No macroscopic changes were observed at necropsy. About the microscopic changes the original report states: "The livers showed enlargement of parenchymal cells in peripheral sectors, together with loss of cytoplasmic basophilia; there were also isolated cell necroses and frequent mitoses. These changes occurred from 9 mg/kg onwards, but were encountered only in a few animals and only to a very slight extent; they did not increase with higher doses and were not sex-related." The changes were slight and infrequent. Therefore it is doubtful how these effects are related to the test substance and whether they are toxicologically relevant. These slight effects in the liver were not found in the first study using higher doses. A third study, though in adequate in some aspects, also reported absence of histological effects (**Dikshith et al. 1988**).

Table A2: Endosulfan; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex/Group size		Males/ 6						Females/ 6				
Dose (mg/kg bw/day)		0	1	3	9	27	81	0	1	3	9	27[1]
Mortalities		0	0	0	2	0	3	0	0	0	0	5
Spleen weights	(g)	0.50	0.58	0.55	0.41	0.53	0.52	0.36	0.43	0.41	0.46*	0.44
	(% bw)	0.17	0.19	0.18	0.16	0.18	0.18	0.17	0.20	0.19	0.21*	0.19
Erythrocyte ChE	(U/l)	209	222	206	222	218	218	192	180	180	202	181
Serum ChE	(U/l)	348	306	296	97*	96*	72*	472	436	381	291	285
Brain ChE	(U/kg)	4438	4153	3802*	3490*	3181*	3357*	3552	3056*	2916*	3065*	3174

[1] Due to one survivor only it was not possible to establish statistical significance in this group.
Significantly different from control, *p<0.05, **p<0.01, ***p<0.001

Conclusion: The LOAEL of 27 mg/kg bw/day for female rats and of 81 mg/kg bw/day for male rats in this 30-day dermal study are based on death observed at these doses. The slight serum cholinesterase inhibition in the males is not considered to be of toxicological significance. From sensitive *in vitro* data it is well known that endosulfan is not a cholinesterase inhibitor. The slight, non-dose-related, brain cholinesterase inhibition in males and females is probably not substance related, since no corresponding changes were found in pharmacologically closely

related erythrocyte cholinesterase activity. Moreover, these changes were not found in another subchronic dermal study using higher dermal doses. Therefore these changes must be considered as a spontaneous finding. Two male deaths at 9 mg/kg cannot be considered as treatment related due to developmental disturbances originating prior to treatment. Therefore, the NOAEL for female rats in this study was 9 mg/kg and for male rats between 27 and 81 mg/kg bw/day.

APPENDIX 3

Dikshith, T.S.S.; Raizada, R.B.; Kumar, S.N. (1988); Effect of repeated dermal application of Endosulfan to rats; Generated by: Ind. Toxicol. Res. Cent., India; Vet. Human Toxicol. Vol. 30, 219 - 224 (1988); Company file No: A41365; Date: 15 Feb. 1988

GLP: The publication was not conducted according to GLP.
Guidelines: The publication does not state that the study was carried in compliance with any guideline.
Information on the methods is scanty and results are sometimes inconsistent. Therefore, the study is **inadequate**.
Materials and Methods: Groups of 6 male and 6 female Wistar rats were painted daily with dermal applications of endosulfan (origin unspecified) in acetone to the shaved lateral abdominal skin for 30 days. Males were dosed at 18.75, 37.50, 62.50 mg/kg bw/day, females at doses of 9.83, 19.66, 32.00 mg/kg. A control group was skin painted with peanut oil. After this period animals were killed by decapitation. At necropsy organs were weighed, enzyme activities in liver and serum measured, and standard hematology was conducted. Macroscopic and histopathological examination was carried out on liver, kidneys, spleen, brain, testes, epididymis, adrenals, ovary and cervix. Residue analysis was carried out in liver, kidney, testes, brain, fat and blood.
Findings:
Mortality: None of the dosed animals died.
Clinical signs: Hyper excitation, tremors, dyspnea and salivation were seen initially. These signs had resolved within one week. The doses at which these signs were observed were not specified.
Body and organ weights: No changes were observed in body weights or relative organ weights.
Hematology: No significant hematological changes were found in female rats. Inconsistent changes were found in the males. Hemoglobin was slightly reduced in all male groups. The number of leukocytes was increased at 62.5 mg/kg and decreased at 37.5 mg/kg. At the latter dose the percentage of lymphocytes had increased and neutrophils reduced. These effects can be dismissed due to the minimal size of the changes or the lack of dose relationship.
Biochemistry: Liver serum GPT activities were significantly decreased. Liver GOT was also significantly decreased but serum GOT was increased. These changes were not dose-related. Various other inconsistent changes were measured.

Table A3: Endosulfan; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex	Males				Females			
Dose (mg/kg bw/day)	0	18.75	37.5	62.5	0	9.83	19.66	32.0
Hemoglobin (g/100 ml)	16.9	16.2*	15.6*	15.8*	16.5	17.0	17.0	16.0
White Bl. Cells ($10^3/mm^3$)	7775	7025	6900*	8425*	8080	8145	8100	8000
Lymphocytes (%)	76	75	86*	76	83	85	86	81
Neutrophils (%)	23	24	13*	22	17	13	12	15
Liver GOT (µmol/g/min)	14.8	9.7*	5.6*	9.1*	8.4	5.4*	6.7	5.4*
Liver GPT (µmol/g/min)	37.0	14.8*	15.2*	14.7*	21.6	14.8*	15.1*	11.2*
Liver Protein(mg/g tissue)	138	123	109*	117*	152	162	186*	195*
Serum GOT (nmol/g/min)	55	72*	69*	59	38	27	54	84*
Serum GPT (nmol/g/min)	102	77*	32**	83	86	47*	67*	49*
Serum AP (nmol/g/min)	396	776*	1095**	730*	505	639	567	539
Serum Protein (mg/ml)	105	109	118	107	85	101*	105*	117**

Significantly different from control, *p<0.05, **p<0.01, ***p<0.001

Histology: No gross or microscopic abnormalities were found in the major organs.
Residue analysis: Residue levels in organs of males in decreasing order were: fat, kidneys, testes, blood, liver, brain; and in organs of the females: fat, liver, blood, kidneys, brain. Residues in females were much higher than in males. Details about the method are not given and the results are inconsistent with the other metabolism studies.
Conclusion: In this 30-day dermal study endosulfan caused transient neurological effects only in the first week. This points to adaptation e.g. by induction of detoxifying enzymes. Biochemical parameters in liver and serum were changed in a non-dose related fashion. In absence of significant biological effects (including histological effects) the NOAEL was probably equal to the highest doses applied.

APPENDIX 4

Thevenaz, Ph.; Luetkemeier, H.; Chevalier, H.J.; Vogel, W.; and Terrier, Ch. (1988); Endosulfan – Emulsifiable Concentrate (Code: HOE 002671 OI EC34 A101). Subchronic (4-Week) Repeated Dose Dermal Toxicity Study in Rats; Research & Consulting Co. AG, Switzerland; Company file No: A39279; Date: 4 Oct. 1988; EPA MRID 41048505

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Method B.9 on Repeated dose (28 days) toxicity (dermal), with OECD Test Guideline 410 (May 1981) and with EPA-FIFRA §82-2 (November 1982).
Materials and Methods: Groups of 15 male and 15 female Wistar rats were treated dermally with 2 ml/kg bw of endosulfan emulsifiable concentrate (33.3% technical in carboxymethylcellulose) for 6 hours/day, 5 days/week for a total of 21-22 applications. There were two control groups, the first received vehicle alone (aqueous 4% carboxymethylcellulose solution), and the second group received formulation base (HOE 002671 OI EC00 A302, administered in vehicle). Male rats were dosed 27, 54 and 81mg/kg bw/day, females at doses of 9, 12, 18 and 36 mg/kg bw/day. After the 6-hour treatment period the bandage was removed and the treated skin was rinse with lukewarm water and dried with a disposable paper towel. Mortality was recorded twice daily and clinical signs were recorded at least once daily. Examination of the treated skin area was done before the next application, food consumption and body weights were recorded weekly. At day 31 the animals were sacrificed and standard hematology, clinical chemistry, urinalysis, macroscopic pathology and histology was carried out.

Findings:
Mortality: One female (no. 131) at 12 mg/kg bw/day and one female (no. 145) at 18 mg/kg bw/day were found dead during treatment week 4. Four females (nos. 151, 152, 160 and 165) at 36 mg/kg bw/day were found dead during treatment weeks 1, 2 and 4. The incidence of mortality in females in the high dose group was considered treatment-related, though no pathomorphological lesions could be distinctly attributed to administration of the test article. One male (no. 11) from vehicle control died following anesthesia for blood sampling during week 3 of recovery period. One female (no. 146) at 18 mg/kg bw/day died following blood sampling during treatment week 4. Neither of these deaths was attributed to treatment.
Clinical signs: Transient signs ranging from slight to severe in intensity were observed after the end of the application period: tremor, Straub-tail, trismus, saltatory spasms, extension spasms, tetanoid spasms. Onset occurred within one hour after daily application, the duration did not exceed approximately 30 minutes. Occurrence was limited to males in the 81 mg/kg bw/day dose group and females in the 18 and 36 mg/kg bw/day dose groups, with isolated cases recorded in females at 12 mg/kg bw/day. One female in the 36 mg/kg bw/day dose group died following one spasm attack. No comparable signs were observed in during the recovery period.
Irritation: In the formulation base control group, erythema of minimal to moderate intensity was observed during both treatment and recovery periods. Mean erythema values increased towards the end of the treatment period, with concomitant slight edema from week 3 of the study. Edema

was not detectable from week 2 of recovery on, and erythema decreased from moderate to marginal mean values over the duration of the treatment-free period. During the second half of the treatment period, marginal to slight erythema was observed in dosage groups 3 to 6, with concomitant marginal edema occurring in groups 4 and 5. During the recovery period, marginal erythema persisted during week 1, whereas edema was no longer detectable. No effects were noted in the vehicle control group.
Body and organ weights: No changes were observed in body weights or relative organ weights.
Hematology: No toxicologically significant hematological changes were found in male or female rats.
Biochemistry: Slightly increased levels of alkaline phosphatase activity a decrease in albumin to globulin ratio was seen in high dose females. Slightly increased aspartate aminotransferase activity was noted in the high dose males. Slightly decreased albumin concentrations were seen in the 18 and 36 mg/kg bw/day dose groups for female rats. Plasma cholinesterase activity was slight decreased (by 22-32%) for females at 12, 18 and 36 mg/kg bw/day.
Histology: Other than skin effects resulting from irritation, no gross or microscopic abnormalities were found in the major organs.

Table A4: Endosulfan 3 EC; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex	Males					Females					
Dose (mg/kg bw/day)	0	0	27	54	81	0	0	9	12	18	36
Mortalities	1[1]	0	0	0	2	0	0	0	1	2[1]	4
Erythrocyte ChE (umol-SH/ml)[2]	1.89	1.85	1.64*	1.56*	1.48*	2.02	1.87	1.92	1.76*	1.82	1.69*
Serum ChE (umol-SH/ml)[2]	0.66	0.68	0.70	0.72	0.70	3.07	2.58	2.56	2.40*	2.19*	2.08*
Brain ChE (umol-SH/g)[2]	5.98	6.13	6.02	5.03*	6.64*	7.63	6.91*	7.32	6.73*	6.95*	6.65*

[1] Non-treatement-related, deaths occurred during blood sampling
[2]Values taken from end of treatment. There were no significant changes following the 4-week recovery period in any dose group.
Significantly different from control, *p<0.05, **p<0.01, ***p<0.001

Conclusion: In this 30-day dermal study endosulfan caused transient neurological effects following each treatment period in the high dose group animals. The NOAEL for systemic toxicity was considered 54 mg/kg bw/day for males and 9 mg/kg bw/day for females. A decrease in erythrocyte cholinesterase was slight, but dose-related in males only at all dose levels.

APPENDIX 5

Ebert, E. (1987) Endosulfan – Water Dispersible Powder (50%)
(Code: Hoe 002671 0I WP50 A501) Subchronic Dermal Toxicity (21 Applications in 30 Days) in Wistar Rats; Hoechst Pharma Research Toxicology, Germany; Company file No: A39426; Date: 17 May 1987. EPA MRID 41048506

GLP: The study was conducted according to GLP.
Guidelines: The study was performed in compliance with EEC Test Method B.9 on Repeated dose (28 days) toxicity (dermal), with OECD Test Guideline 410 (May 1981) and with EPA-FIFRA §82-2 (November 1982).
Material and Methods: Groups of 11 male and 11 female Wistar rats were treated dermally with 2 ml/kg bw of endosulfan WP (purity 49.5%) in the form of 2 – 32% aqueous dispersions under total occlusion 6 hours/day, 5 days/week (21 treatments in total) for 30 days. Dosages were 0, 40, 80 (females only), 160 and 640 mg/kg bw/day (males only). After the 6-hr treatment the bandage was removed and the treated skin washed. Behavior and clinical signs were recorded daily, examination of the treated skin area was done before the next application, food consumption and body weights were recorded twice weekly, water consumption once weekly. At day 31 the animals in the main group (6/sex/dose) were sacrificed and standard hematology, clinical chemistry, urinalysis, macroscopic pathology and histology was carried out. A recovery group of 5/sex/dose were sacrificed on days 23 or 24 after termination of the treatment.
Findings: The main findings are summarized in Table A.
Mortality: Mortalities occurred between day 2 – 24 in the females only. Three animals in the highest dose group (160 mg/kg bw/day) dies on day 3, 11 and 24, respectively. None of these animals showed previous clinical signs of intoxication. One female in the 80 mg/kg bw/day group died day 21.
Clinical signs: One female in the 80 mg/kg bw/day group showed dacryohemorrhea and a blood-crusted snout on day 22 of the study. No clinical signs of intoxication or mortality were noted at any dose in the male rats. No signs of neurological disturbance, changes in the eyes, damaged to the oral mucosa or impairment of dental growth were observed in any of the treatment groups.
Irritation: At the end of the first and during the second week of treatment, there was a slight redness of the skin in individual animals from the 80 mg group and in many of the animals from the 160/640 mg groups. Dry and chapped skin was noted in the high dose animals with fine or course scales. These signs of irritation receded by the end of the second week, and were only present in a few individual animals during the third week of the study.
Body weights: From the second week of treatment onwards, males in the 640 mg/kg bw/day group showed significantly reduced body weight gains when compared to controls. No other dose group showed significant weight changes.
Food consumption: There was a marked reduction in food intake
Water consumption: There was a marked reduction in water intake
Hematology: No hematological effects were found.
Clinical chemistry: Cholinesterase activity was reduced in serum of females treated at 80 and 160 mg/kg bw/day. This effect, which was discernible as a tendency after the end of the

recovery period, was possibly due to reduced biosynthesis of the enzyme in the liver and could be interpreted as an impairment of hepatic function. There were no indication s of cholinesterase inhibition, since a comparable effect of the substance on erythrocytes and brain ChE was not observed. There was also a slight increase in cholesterol and total lipids in the high dose females. All other serum clinical chemical parameters were also within normal range
Organ weights: All organ weights were within a normal range of biological variation.
Urinalysis: No changes were found.
Macroscopic pathology or histology: No macroscopic or microscopic changes were observed at necropsy.

Table A5: Endosulfan 50WP; 30-Day Dermal Toxicity Study in the Wistar Rat

Sex/Group size	Males/6				Females/6			
Dose (mg/kg bw/day)	0	40	160	640	0	40	80	160
Mortalities	0	0	0	0	0	0	1	3
Erythrocyte ChE (U/l)	332	424	356	379	514	507	486	548
Serum ChE (U/l)	467	407*	426	405*	1318	1005	952*	709*
Brain ChE (U/kg)	4267	4538	4579	4891*	4173	4257	3944	4074

Significantly different from control, *p<0.05, **p<0.01, ***p<0.001

Conclusion: The LOAEL of 80 mg/kg bw/day for female rats and of 160 mg/kg bw/day for male rats in this 30-day dermal study are based on death observed at these doses. The slight serum cholinesterase inhibition in the females is not considered to be of toxicological significance. From sensitive *in vitro* data it is well known that endosulfan is not a cholinesterase inhibitor.

Appendix 6

CONSIDERATION OF SKIN PENETRATION FOR RISK ASSESSMENT IN MAN

For risk assessment in man the dermal absorption (percent) for a 8-10 hour exposure period is normally selected since it reflects an average working day for the pesticide handlers (mixer/loader/applicator). In addition, interspecies differences (experimental animal to man) in skin penetration have to taken into consideration

In vivo dermal penetration study in the rat

For extrapolation of animal data to the human situation, an *in vivo* dermal penetration study in the rat has been conducted (MRID 41048504).
Groups of 16 female Sprague Dawley rats were exposed dermally for 10 hours to a similar-to-field-use 3 EC formulation of ^{14}C-endosulfan on a circular area of shorn dorsal skin at actual levels of 0.09, 0.98 or 10.98 mg ^{14}C-endosulfan /kg body weight. Thereafter the skin was thoroughly washed with a mild soap solution to remove non-absorbed substance. After 24, 48, 72 and 168 hours 4 animals/group were sacrificed and disposition of ^{14}C was measured.
Based on the results of this study the absorption of endosulfan in a typical formulation by the skin of rats goes fairly rapid and is concentration dependent. The dermal penetration of endosulfan amounts to 20% at a high dose of 10 mg/kg after a 10hr dermal exposure. At lower doses the penetration is lower in absolute terms, but higher in terms of percentage, increasing to just over 40%.
However true penetration, i.e. release from the skin into the blood, takes a long time and is the rate-limiting factor. After 24 hours 80% or more of absorbed material was still bound to the skin of rats, while most of the penetrated material (1 - 10%) had been excreted. The maximum penetration occurs after 48 hr in good correlation with peak concentrations found in liver and kidneys at that time point. After one week 95% of the absorbed material had penetrated. More details of this study are presented below.

In vitro penetration of endosulfan through rat and human skin

To reduce the uncertainty of the interspecies extrapolation an “in vitro” study comparing the penetration of endosulfan through excised rat and human skin has been performed (MRID 44863701). In this study the rate of penetration of an experimental ^{14}C-endosulfan-3 EC formulation through isolated human and rat skin was assessed *in vitro* following a single application of 1.0, 0.1 or 0.01 mg endosulfan/cm² to the epidermal surface. Penetration was measured by assessment of radioactivity in duplicate aliquots of receptor-fluid after 1, 2, 4, 8, 10, 16, 24, 48, and 72 hours.
Based on the results of this study the dermal penetration rate through rat skin was a 3.1 – 5.7 times higher than that observed in human skin. More details of this study are presented in below.

Craine, Elliott M. (1988); A Dermal Absorption Study in Rats with ^{14}C-Endosulfan with Extended Test Duration; Wil Research Laboratories Inc. Ohio, USA; Company file No: A39677; Date: 17 November 1988. EPA MRID 41048504

GLP: The study was conducted according to GLP.
Guidelines: The study complies with Test Guideline EPA-FIFRA § 85-3.
Material and Methods : Groups of 16 female Sprague Dawley rats were exposed dermally for 10 hours to a similar-to-field-use 3 EC formulation of ^{14}C-endosulfan on a circular area of shorn dorsal skin at actual levels of 0.09, 0.98 or 10.98 mg ^{14}C-endosulfan /kg bw. Thereafter the skin was thoroughly washed with a mild soap solution to remove non-absorbed substance. After 24, 48, 72 and 168 hours 4 animals/group were sacrificed and disposition of ^{14}C was measured.
Findings : No signs of systemic intoxication or any signs of skin irritation were observed. Results are summarized in Table A4. Skin absorption was proportional to the dose, but was in all doses less than 50% and at the high dose (10.98 mg/kg) was only 20%. Only a small amount of residue was still present in the skin after one week, indicating that the penetration process was completed in one week. Some residue also remained in other parts of the body. Concentrations in organs were highest in kidneys and liver and had reached a peak after 48 hours, at which time point the penetration rate had also reached its maximum. Peak concentrations in fat were only half of those in the liver and dissipated much more quickly. The rate of elimination of ^{14}C-material was low at 24 hr, then accelerated with a peak at 48 hours and subsequently slowed down again. Two thirds of the eliminated radioactivity was excreted in the feces and one third in the urine.

Table A6: Disposition of endosulfan in the rat after dermal application

Measured Dose (mg/kg)		0.09	0.98	10.98
Not absorbed	(%)	39.9	54.7	71.8
Absorbed	(%)	46.5	48.0	21.3
Present in skin	(%)	1.7	1.5	1.0
Total penetrated	(% after 24hr)	22.1	16.1	3.8
	(% after 48hr)	35.3	36.2	11.1
	(% after 72hr)	39.0	28.7	12.0
	(% after168hr)	44.8	46.4	20.3
Present in animal	(%)	2.5	2.3	1.3
Excreted Feces	(% after 24hr)	5.6	3.2	0.6
	(% after 48hr)	14.8	13.6	3.2
	(% after 72hr)	21.9	15.2	5.6
	(% after168hr)	28.6	31.1	13.2
Excreted Urine	(% after 24hr)	3.5	2.7	0.7
	(% after 48hr)	7.9	7.2	2.4
	(% after 72hr)	10.4	7.2	2.8
	(% after168hr)	13.7	13.1	5.8
Excreted Total	(% after 24hr)	9.0	5.8	1.4
	(% after 48hr)	22.6	20.6	5.6
	(% after 72hr)	32.4	20.9	8.4
	(% after168hr)	42.3	44.1	19.1
Max. penetration rate	(µg/cm^2/hr)	0.018	0.165	0.532
Total Recovery	(%)	86.4	102.7	93.1

Conclusion: Absorption of endosulfan in a typical formulation by the skin of rats goes fairly rapid and is concentration dependent. The dermal penetration of endosulfan amounts to 20% at a high dose of 10 mg/kg after a 10hr dermal exposure. At lower doses the penetration is lower in absolute terms, but higher in terms of percentage, increasing to just over 40%.
However true penetration, i.e. release from the skin into the blood, takes a long time and is the rate-limiting factor. After 24 hours 80% or more of absorbed material was still bound to the skin of rats, while most of the penetrated material (1 - 10%) had been excreted. The maximum penetration occurs after 48 hr in good correlation with peak concentrations found in liver and kidneys at that time point. After one week 95% of the absorbed material had penetrated.

Noctor, J. C.; John, S. A. (1995). (^{14}C)-Endosulfan; Rates of penetration through human and rat skin determined using an in vitro system; Hazleton Europe, Harrogate, United Kingdom; Company file No: A54103; Date: April 1995. EPA MRID 44863701

GLP: The study was conducted according to GLP.
Guidelines: The study complies with the draft OECD Guideline for 'Percutaneous Absorption: *in vitro* Method'.
Material and Methods: The rate of penetration of an experimental ^{14}C-endosulfan-3 EC formulation through isolated human and rat skin was assessed *in vitro* following a single application of 1.0, 0.1 or 0.01 mg endosulfan/cm² to the epidermal surface. These doses are of the same order as those used for the described above *in vivo* dermal penetration studies on rats. Frozen intact skin was thawed, cut to uniform thickness resulting in sections of 400 µm. The resulting section consisted of intact epidermis and a portion of dermis. After a check for membrane integrity, a nominal application volume of 64 µl test substance in aqueous solution was added to eight skin sections per dose (12 in the high dose), each mounted in a 'Franz' static *in vitro* dermal penetration cell. Penetrated material was collected in receptor fluid under the skin. This fluid consisted of acidified ethanol/water (1:1) and was held at a temperature of 32°C. Penetration was measured by assessment of radioactivity in duplicate aliquots of receptor-fluid after 1, 2, 4, 8, 10, 16, 24, 48, and 72 hours. After 72 hours the epidermal surface of all skin-preparations was thoroughly washed with mild detergent. The wash was collected and its radioactivity measured to assess the amount of non-absorbed material. The 4 additional skin-preparations in the high dose groups were given an additional wash after ten hours. Radioactivity in skin was also measured to assess material absorbed but not penetrated. Dermal metabolism was assessed by analysis of metabolites in the receptor fluid.

Table A6.1: ^{14}C-Endosulfan 3 EC Formulation; Comparative 72 hr skin penetration model

Dose (mg/cm²)		0.01		0.1		1.0		1.0 (10hr wash)	
Species		Rat	Man	Rat	Man	Rat	Man	Rat	Man
% not absorbed	(72 hr)	1.7	26	3.9	44	23	7	51	59
% present in skin	(72 hr)	13.3	7	14.3	13	30	49	28	5
% penetrated	(72 hr)	96	61	76	29	40	20	9	4
% penetrated	(10 hr)	79	18	35	6	13	7	12	2
% total recovery	(72 hr)	111	94	94	87	95	76	88	68
Penetration rate (1-8hr µg/cm²/hr)		0.9	0.2	4.4	0.8	15.9	5.2	15.9	5.2
Penetration Ratio Rat/Man		4.0		5.7		3.1		3.1	

Findings: Penetration of endosulfan through the skin started after a lag time of generally less than one hour at a steadily decreasing rate. The penetration rate was highest between 1 - 8 hr. The penetration rate was dose-dependent and found to be on average 4.3 times higher in rat skin than in human skin. For results see Table A6.1 above. Analysis of the receptor fluid in the high dose groups further revealed interesting differences in degradation products, showing that human skin has more residual detoxifying capacity than rat skin. This analysis has been summarized below in Table A6.2.

Table A.6.2: ^{14}C-Endosulfan 3 EC Formulation; Degradation Products in Receptor Fluid after 72hr

Metabolite	Rat*	Human*
α-Endosulfan	3.1	-
β-Endosulfan	81.4	27.3
Endosulfan-sulfate	2.9	8.3
Endosulfan-diol	8.8	34.0
Endosulfan- OH-ether	-	2.7
Unknown	-	17.2

- Figures indicate percentage of the total radioactivity in receptor fluid

Conclusions: The rate of penetration through human skin is significantly lower than through rat skin. The difference is concentration dependent and the mean ratio rat/man is 4.3. The results of this *in vitro* study are consistent with the results of the *in vivo* dermal penetration study on rats. The skin appears to have a depot function, slowing down the rate of penetration significantly. Longer storage time in the skin may be the basis of the higher degree of dermal detoxification.

Appendix 7

Dikshith T.S.S. et al. (1988)

Effect of Repeated Dermal Application of Endosulfan to Rats

Reprinted from Veterinary and Human Toxicology, Vol. 30, No. 3, June 1988, pp. 219-224

(Full Paper)